Brexpiprazole II: Antipsychotic-Like and Procognitive Effects of a Novel Serotonin-Dopamine Activity Modulator

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ABSTRACT

Brexpiprazole (OPC-34712, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy}quinolin-2(1H)-one) is a novel serotonin-dopamine activity modulator with partial agonist activity at serotonin 1A (5-HT1A) and D2A receptors, combined with potent antagonist effects on 5-HT2A, α1B-, and α2C-adrenergic receptors. Brexpiprazole inhibited conditioned avoidance response (ED50 = 6.0 mg/kg), amphetamine- or α-amanitamine-induced hyperactivity (ED50 = 2.3 and 0.90, respectively), and amphetamine-induced stereotypy (ED50 = 2.9) in rats at clinically relevant D2 receptor occupancies. Brexpiprazole also potently inhibits apomorphine-induced eye blinking in monkeys. The results suggest that brexpiprazole has antipsychotic potential. Brexpiprazole induced catalepsy (ED50 = 20) well above clinically relevant D2 receptor occupancies, suggesting a low risk for extrapyramidal side effects. Subchronic treatment with phencyclidine (PCP) induced cognitive impairment in both novel object recognition (NOR) and attentional set-shifting (ID-ED) tests in rats. Brexpiprazole reversed the PCP-induced cognitive impairment in the NOR test at 1.0 and 3.0 mg/kg, and in the ID-ED test at 1.0 mg/kg. However, aripiprazole (10 mg/kg) was ineffective in both tests, despite achieving relevant D2 occupancies. In the NOR test, the 5-HT1A agonist buspirone and the 5-HT2A antagonist M100907 [(R)-(2,3-dimethoxyphenyl)-1-(4-fluorophenethyl)piperidine-4-yl]methanol partially but significantly reversed PCP-induced impairment. Furthermore, the effect of brexpiprazole was reversed by cotreatment with the 5-HT1A antagonist WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate). The results indicate that brexpiprazole has antipsychotic-like activity and robust efficacy in relevant models of cognitive impairment associated with schizophrenia. The effects of brexpiprazole in the cognitive tests are superior to those of aripiprazole. We propose that the pharmacologic profile of brexpiprazole be based on its balanced effects on 5-HT1A, D2, and 5-HT2A receptors, with possible modulating activity through additional monoamine receptors.

Introduction

The main strategy for treatment of schizophrenia is based on functional dopamine antagonism. In addition to D2 receptor antagonism, almost all second-generation antipsychotics include antagonism of serotonin 2A (5-HT2A) receptors and of α1-adrenoceptors. Some compounds also affect a variety of other monoamine receptors, such as 5-HT1A/6/7 receptors, α2-adrenoceptors, and histamine and muscarinic receptors. The broad target effects are aimed at either improving efficacy (e.g., potential effects on affective symptoms or cognitive deficits) or mitigating adverse effects related to the central nervous system (CNS), such as extrapyramidal symptoms (EPS), or to the endocrine system, such as hyperprolactinemia (Arnt and Skarsfeldt, 1998; Roth et al., 2004; Amt et al., 2008; Newman-Tancredi, 2010; Newman-Tancredi and Kleven, 2011).

ABBREVIATIONS: 5-HT, serotonin; CAR, continued avoidance response; CI, confidence interval; CNS, central nervous system; ED, extradimensional; EDR, extradimensional reversal; EPS, extrapyramidal symptoms; ID, intradimensional; ID2R, intradimensional reversal task; ID-ED, attentional set-shifting; LE, Long Evans; LH, Lister hooded; M100907, (R)-(2,3-dimethoxyphenyl)-1-(4-fluorophenethyl)piperidine-4-yl)methanol; MK-801, (5S,10R)-(-)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; ORM-10921, (1S,12bS)-1-(methoxyethyl)methyl-2,3,4,6,7,12b-hexahydro-1H-benzofuro[2,3-a]quinolizine; SB-269970, (R)-3-[2-[4-(4-methylpiperidin-1-yl)ethyl]pyrroloidin-1-ylsulfonyl]phenol; SB-271046, 5-chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[2,3]thiophene-2-sulfonamide hydrochloride; subPCP, subchronic phencyclidine; subVeh, subchronic vehicle; WAY100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate.

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Because of tolerability issues, treatment with D2 antagonists is not considered the optimal strategy to modulate dopaminergic activity, and the discovery and development of D2 partial agonists has provided a well tolerated treatment with stabilizing effects on dopamine function (Stahl, 2001). So far, only one D2 partial agonist, aripiprazole, with moderate D2 intrinsic activity, has reached the market with the approved indications of schizophrenia and bipolar mania and as an add-on treatment of major depression (Fleischhacker, 2005). Another compound with D2 intrinsic activity similar to aripiprazole is in development (e.g., cariprazine; Kiss et al., 2010; Citrome, 2013), but others with higher D2 intrinsic activity have seen their development discontinued (e.g., bifeprunox; Newman-Tancredi et al., 2007) because of their insufficient clinical efficacy (Casey et al., 2008).

A key issue for D2 partial agonism is to determine an optimal level of intrinsic activity (or relative efficacy at the D2 receptors that would lead to a desirable stabilization of dopaminergic transmission). Too high D2 intrinsic activity leads to a lack of robust clinical activity and to adverse effects related to increased D2 receptor tonus, including nausea, vomiting, insomnia, and motor effects, such as hyperkinesias and restlessness (Fleischhacker, 2005; Casey et al., 2008), whereas excessive D2 antagonist activity results in an increased risk for EPS and increased prolactin secretion (Casey, 1996).

In addition to optimizing D2 intrinsic activity, modulating other neurotransmitter systems may contribute to improved efficacy and tolerability. The D2 partial agonist aripiprazole shows 70–90% D2 receptor occupancy at clinically relevant doses, a partial agonist effect at 5-HT1A receptors, and some antagonism at 5-HT2A receptors (Mamo et al., 2007; Dahan et al., 2009). However, the occupancies at 5-HT1A and 5-HT2A receptors are considerably lower than that at D2 receptors in patients with schizophrenia (Mamo et al., 2007), suggesting the receptor profile might not be optimal for clinical efficacy. Accordingly, a broader target profile (e.g., on selected 5-HT receptor and α-adrenoceptor subtypes) may lead to improved clinical efficacy and tolerability in the treatment of schizophrenia, including efficacy on positive symptoms and cognitive deficits, the main factors determining functional outcome in schizophrenia (Green, 1996). A recent meta-analysis study suggested that 5-HT2A antagonism may reduce D2 antagonist–induced akathisia (Laoutidis and Luckhaus, 2014). Furthermore, a broad pharmacologic profile could offer a wider potential in the treatment of a variety of other CNS disorders and symptoms, such as major depressive disorder and anxiety disorders, including post-traumatic stress disorder (Roth et al., 2004; Arnt et al., 2008; Wong et al., 2008).

Brexpiprazole (OPC-34712, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy}quinolin-2(1H)-one) was discovered by Otsuka Pharmaceutical Co Ltd. (Tokyo, Japan) and is being developed in collaboration with H. Lundbeck A/S (Valby, Denmark). It is a novel serotonin-dopamine activity modulator, combining moderate-intrinsic activity 5-HT1A receptor partial agonism and low-intrinsic activity D2 receptor partial agonism with antagonist activity on a variety of 5-HT and α-adrenergic receptor subtypes. Its basic in vitro and in vivo pharmacologic profile has been presented in detail in an accompanying paper (Maeda et al., 2014).

In the present study, the in vivo behavioral pharmacologic characteristics of brexpiprazole are evaluated and compared with two other second-generation antipsychotics, aripiprazole and risperidone, using several preclinical animal models and tests relevant to schizophrenia, including apomorphine/d-amphetamine–induced behavioral disturbances in rats and monkeys, conditioned avoidance response (CAR), catalepsy tests, and subchronic phencyclidine (PCP)–induced cognitive impairments in rats.

### Materials and Methods

#### Subjects

Male Wistar rats (CAR, apomorphine-induced hyperactivity and stereotyped behavior, and catalepsy; 126–200 g at time of testing [Japan SLC Inc., Shizuoka, Japan]; and amphetamine-induced hyperactivity and spontaneous locomotor activity; 150–175 g at time of testing [Charles River, Koln, Germany]), male Lister hooded (LH) rats (novel object recognition [NOR]; 220–240 g at time of testing [Charles River]), and male Long Evans (LE) rats (attentional set-shifting [ID-ED]; 180–280 g at the time of testing [Charles River, Wilmington, MA]) were used. Albino Wistar rats are commonly used for standard behavioral testing of CNS compounds, and the pigmented LH and LE strains are preferred for the cognitive tests. These latter strains have been shown to acquire cognitive tests faster and more reliably (Andrews et al., 1995). For this reason, the NOR test was validated using LH rats (Ist id et al., 2010; Redrobe et al., 2010), and the ID-ED test was validated with either LH (Goetghuer and Dias, 2009) or LE rats (Rodefer et al., 2008). However, only the LE rat strain was available in the People’s Republic of China, so it was selected for use in the ID-ED test. Rats had food and water available ad libitum, except for specified periods in each test. They were housed 2–4 per cage in standard Makrolon type III cages and were maintained on a 12-hour light/dark cycle (lights on 6:00 or 7:00 AM) in environmentally controlled climate conditions.

Male Cynomolgus monkeys (5–7 years old; Japan Wild Animal Research Center Inc., Kagoshima, Japan; apomorphine-induced eye blinking) were also used. Monkeys were housed individually with 200 g of certified primate diet 5048 (PMI Nutrition International, Shoreview, MN) and 600 ml of tap water daily.

The care and handling of rats was in accordance with relevant guidelines: Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co, Ltd. (revised on 1 April 2004), the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act, Code of Federal Regulations Title 9, Chapter 1, Subchapter A for apomorphine-induced behavior, CAR, and catalepsy tests; the Danish Executive Order No. 1306 of November 23, 2007, for NOR, t-amphetamine–induced hyperactivity, and spontaneous locomotor activity tests; the Minister of Health’s 2001 laboratory animal requirements for environment and housing facilities, and the People’s Republic of China (GB 14925-2001) for the ID-ED test. The care and handling of monkeys was in accordance with Japan SLC Inc. Experimental Animal Welfare Policy.

#### Drugs

Brexpiprazole, aripiprazole, and risperidone were synthesized by Otsuka Pharmaceutical Co., Ltd. Apomorphine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO). The t-amphetamine sulfate and buspirone hydrochloride were obtained from Sigma-Aldrich. Modafinil, PCP, and WAY100635 ([N-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate) were synthesized by H. Lundbeck A/S. Apomorphine was dissolved in saline immediately before injection; brexpiprazole, aripiprazole, and risperidone were suspended in 5% gum arabic–distilled water solution for CAR, apomorphine, and catalepsy studies. In cognition tests, t-amphetamine, and spontaneous locomotor activity studies, brexpiprazole and aripiprazole were dissolved in minimum amounts of 1 mM methanesulphonic acid in 10% HP-β-cyclodextrin solution, adjusted to pH 5.0 using 0.1 M NaOH. It was confirmed that there was no major difference in plasma...
concentration of brexpiprazole at 3 mg/kg between the two vehicles (C. Bundgaard, unpublished data on file). Modafinil was suspended in 0.5% methylcellulose, and phencyclidine, d-amphetamine sulfate, buspirone, and WAY100635 were dissolved in saline. Doses of salts are expressed as free bases, except for tool compounds (e.g., apomorphine, d-amphetamine, PCP). Test compounds for in vivo studies were administered by mouth or subcutaneously in a volume of 5 ml/kg, except in monkey studies (1 ml/kg).

CAR in Rats

The shuttle box (dimensions: 46 × 19.5 × 20 cm; Bio Medica, Osaka, Japan) was placed in a sound-attenuated chamber and subdivided into two compartments by a hurdle (elastic band, 1 mm in width, 3 cm in height). The shuttle box floor was made from stainless steel bars (diameter: 4 mm, and spaced at 1.2 cm). On the upper part of both side panels of chambers, small lamps were mounted, and a house buzzer was set in the center of the chamber ceiling. The position of the animal in the shuttle box was detected by a microswitch attached to the tilting floor. Rats were trained to avoid a scrambled electric shock delivered through the grid floor of the shuttle box. On the first day of training, the rats were habituated for 10 minutes to the shuttle box. From the second day, rats were exposed to a daily session of 20 trials for 7 consecutive days.

Each trial consisted of a 10-second warning tone (105-decibel tone) as a conditioned stimulus followed by a 10-second foot shock (1 mA) as an unconditioned stimulus and a 15- to 75-second (mean: 45 second) intertrial interval. The unconditioned stimulus was terminated when the animal jumped over the hurdle from one compartment to the other or after a cutoff time of 10 seconds. Each rat was placed in one of the compartments of the shuttle box and allowed free exploration for 1 minute before starting the trial.

During the training session, three kinds of responses were recorded: crossing in response to a conditioned stimulus alone was recorded as a CAR; crossing during the unconditioned stimulus presentation was recorded as an escape response; failure to react was recorded as an escape failure. When the animal completed 75% correct avoidances (15 CAR/20 trials) for three consecutive training sessions, it was used for evaluating the effects of compounds in test sessions. On the day after the last training day, well-trained animals (4–6 rats per group) were administered the test compounds p.o., 1 hour before the test session, with the exception of aripiprazole, which was given p.o. 2 hours before testing. The test session consisted of 20 trials, and the CAR, escape responses, and escape failures of each animal were recorded. ED50 values with 95% confidence intervals (CI) were calculated by nonlinear regression analysis using SAS software (SAS Institute Japan, Tokyo, Japan).

Apomorphine-Induced Hyperactivity in Rats

Brexpiprazole or aripiprazole were administered p.o. at 1 and 2 hours, respectively, before apomorphine injection (0.25 mg/kg s.c.). Five rats were used in each dose group. Thirty minutes before the apomorphine injection, each rat was placed individually in a plastic circular chamber (diameter: 30 cm × height 30 cm) and acclimated to the new environment. Measurement of locomotor activity was counted over 1 hour starting immediately after the apomorphine injection. The locomotor detection system (Yamashita Giken, Tokushima, Japan) was composed of a fixed pivot at the center of each chamber with six microswitches fitted beneath the perimeter to detect turning movement as the animal passed over them. The apomorphine dose was selected to induce the maximum level of locomotor stimulation while avoiding the stationary stereotyped behaviors that emerge at higher doses (see the following section) (Costall and Naylor, 1973; Arnt et al., 1988; Kikuchi et al., 1995). The ED50 values with 95% confidence intervals were calculated by nonlinear regression analysis using SAS software.

Apomorphine-Induced Stereotyped Behavior in Rats

Rats were fasted for 16–20 hours before the administration of test compounds. Brexpiprazole and risperidone were administered p.o. 1 hour before the apomorphine injection (0.7 mg/kg s.c.). Aripiprazole was administered p.o. 2 hours before the apomorphine injection. Six rats were used in each dose group. To habituate them to the test environment, each rat was placed individually in an acrylic cylinder (diameter: 23 cm × height 30 cm) 30 minutes before the apomorphine injection.

Stereotyped behavior was recorded by an observer blinded to the treatment groups (compound and dose), for a 1-minute interval every 10 minutes over the 20- to 40-minute period after the apomorphine injection. The total score for the three observations was calculated using the following scoring scale: 0, the appearance of the animals was the same as drug-naive rats; 1, discontinuous sniffing, constant exploratory activity; 2, continuous sniffing, periodic exploratory activity; 3, continuous sniffing, discontinuous biting, gnawing, or licking, and very brief periods of locomotor activity; 4, continuous biting, gnawing, or licking and no exploratory activity (Costall and Naylor, 1973; Arnt et al., 1988; Kikuchi et al., 1995). The dose of apomorphine was selected to induce an average behavioral score of 3 to 4 in the control groups. ED50 values with 95% CI were calculated by nonlinear regression analysis using SAS software.

Amphetamine-Induced Hyperactivity in Rats

Locomotor activity in rats was measured using activity boxes equipped with photocells sensitive to infrared light. The activity boxes (Makrolon type III cage, high model) were equipped with four infrared light sources and photocells placed 4 cm above the floor. The locomotor activity was quantified by counting the number of photobeam interruptions. Re-}

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pretreatment with brexipiprazole. The measurements were conducted according to the literature (Migler et al., 1993; Kleven and Koo, 1996).

Brexipiprazole or vehicle was administered 4 hours before apomorphine-injection through a catheter (8 French, length 40 cm), inserted through the nose into the stomach, using a volume of 1 ml/kg. Apomorphine (0.16 mg/kg) was injected intramuscularly into the femoral region. The number of eye blinks was counted over a 1-minute period, 5, 15, and 30 minutes after saline or apomorphine injection, by two observers blinded to the treatment administered. Counts at each time point were averaged, and the mean numbers of three time points were summed and expressed as the total score. Statistical significance between spontaneous and apomorphine-induced eye blinking was analyzed by two-tailed paired t test, and the ED₅₀ value with 95% CI was calculated by nonlinear regression analysis using SAS software.

**NOR in Rats Treated with Subchronic PCP**

**Test Protocol.** NOR testing was performed as described by Redrobe et al. (2010). In brief, animals were habituated to the test arena (dimensions: 95 × 45 × 50 cm) for 10 minutes on day 1. On day 2, individual animals were introduced to the arena for an acquisition session with two identical objects (two opaque Perspex pyramids, 10 × 10 × 6 cm, or two domed glass paperweights, 8 × 8 × 8 cm) for 3 minutes. The animals were then transferred to their home cages for a 1-hour intertrial interval. After this period, the animal was reintroduced into the arena for the test session. Here an object identical to the familiar objects used in the acquisition, as well as a novel object, were placed in the arena, and the animal was allowed to explore during a 3-minute test session. Behavior of rats was recorded by video, and object interaction was scored manually by an observer blinded to the treatment groups. Object exploration was defined as sniffing, licking, or touching the object while facing it. Animals were excluded from the data analysis if they did not reach both of the following criteria during the retention trial: 1) a total exploration time of 15 seconds or above, and 2) a minimum of 2-second exploration time on each object. Treatment groups/subjects and novel/familiar object identity and location were randomized across the experiment.

**Drugs and Treatment Schedule.** Rats received subchronic PCP (subPCP, 5 mg/kg i.p.) or saline (subchronic [subVeh], i.p.) twice daily at 7:00 AM and 7:00 PM for 7 days, followed by a washout period of 8–9 days before behavioral testing. On the test day, brexipiprazole, aripiprazole, or vehicle was administered p.o. 2 hours before the acquisition trial. M100907 ([R]-2,3-dimethoxyphenyl)[1-(4-fluoro-10]-(2,3-dimethoxyphenyl)piperidin-4-yl)methanol], buspirone, WAY100635, or saline were administered subcutaneously 30 minutes before the acquisition trial. The number of rats in each group is indicated in Figs. 1–3.

**Data Analysis.** Results are presented as 1) exploration time(s) of novel or familiar object and 2) discrimination index, calculated as the novel object exploration time (Tn) minus the familiar object exploration time (Tf) divided by the total exploration time (Tf + Tn).

**Statistics.** Paired t test was used to analyze exploration time. One-way analysis of variance followed by appropriate multiple comparison versus control post-hoc analysis (Bonferroni) was used to investigate statistical discrimination index and total exploration differences between test groups (P < 0.05).

**Exposure.** Blood samples were drawn from animals treated with test compounds after completion of the NOR assay (n = 6 per test compound group). Drug concentrations were determined in plasma using ultraperformance liquid chromatography followed by tandem mass spectrometry detection in positive-ion electrospray ionization mode. Brain homogenate was prepared by homogenizing the brain 1:4 (v/v) with water-2-propanol: dimethyl sulfoxide (50:30:20 v/v/v) followed by centrifugation and collection of the supernatant. Plasma and brain supernatant samples were frozen at −80°C until analysis.

**Fig. 1.** Effects of brexipiprazole (BREX) and aripiprazole (ARI) on (A) exploration time(s) of familiar and novel objects and (B) discrimination index in a NOR task in rats treated with subchronic PCP. Brexipiprazole and aripiprazole were administered p.o. 2 hours before acquisition trial. Data represent mean ± S.E.M, n = 11 (subVeh/vehicle), n = 11 (subPCP/vehicle), n = 10 (brexipiprazole, 0.3 mg/kg), n = 10 (brexipiprazole, 1.0 mg/kg), n = 6 (brexipiprazole, 3.0 mg/kg), n = 8 (aripiprazole, 10 mg/kg). (A) **P < 0.01; ***P < 0.001 versus familiar object exploration. (B) *P < 0.05 versus subVeh/vehicle group; **P < 0.01; ***P < 0.001 versus subPCP/vehicle group.

**Attentional Set-Shifting in Rats Treated with Subchronic PCP**

This test was conducted according to Goetghebeur and Dias (2009) and Goetghebeur et al. (2010), which is a modified version of the protocol described by Birrell and Brown (2000).

**Apparatus.** The test apparatus (dimensions: 44 × 64 × 30 cm) consisted of a three-compartment black box (a holding area and two choice compartments). A terra cotta pot (diameter: 11 cm), recessed into the floor of the test box, was placed in each choice area. Digging medium cues (e.g., HAMA plastic beads, paper confetti, paper clips, wall plugs) were added to the pots, odor cues (oils from The Body Shop International, London, UK) were applied around the rim of each pot, and all were novel to the rats.

**Drugs and Treatment Schedule.** Rats received subPCP (5 mg/kg i.p.) or subVeh (saline, i.p.), twice a day at 8:00 AM and 8:00 PM for 7 days, followed by a 7-day washout period before behavioral testing. Brexipiprazole and aripiprazole were administered subcutaneously 1 hour before test. Modafinil (64 mg/kg) was administered p.o. twice,
each at 32 mg/kg, once 30 minutes before the simple discrimination task and again 30 minutes before the fifth (intradimensional reversal task) discrimination stage because of its short plasma half-life in rats.

**Behavioral Testing.** On days 4–6 of the washout period, rats were habituated to the arena and learned to dig in test pots filled with cage bedding and food rewards (Honey Loops cereal; Kellogg’s, Warrington, UK). On the final day of the washout period (day 7), all rats were presented with two different media and, thereafter, two different odors and were required to learn which of two media or two odors were associated with the food reward. On the day after the 7-day washout period, food-deprived rats were presented with a series of seven discrimination tasks: simple discrimination, compound discrimination, intradimensional shift 1 (ID1), intradimensional shift 2 (ID2), extradimensional reversal task (EDR), and extradimensional reversal task (EDR). Rats only progressed from one discrimination task to the next (always presented in the same order) after reaching a criterion performance level of six consecutive correct responses. Rats were allowed one "discovery" trial at the start of each training or test discrimination task, in which they were allowed to self-correct a dig in the wrong pot.

The number of trials to reach criterion performance was recorded for each of the seven discrimination tasks presented (simple discrimination, compound discrimination, ID1, ID2, ID2R, ED, and EDR). Omissions were defined as an animal’s refusal to participate in the task for more than 15 consecutive minutes. After an omission, animals were returned to their home cage to rest for approximately

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**Fig. 2.** Effects of M100907 and buspirone in comparison with brexpiprazole (BREX) on (A) exploration time(s) of familiar and novel objects and (B) discrimination index in a NOR task in rats treated with subchronic PCP. M100907 and buspirone were administered subcutaneously 30 minutes before acquisition trial. Brexpiprazole was administered p.o. 2 hours before acquisition trial. Data represent mean ± S.E.M., n = 12 (subVeh/vehicle), n = 11 (subPCP/vehicle), n = 12 (subPCP/brexpiprazole, 3.0 mg/kg), n = 9 (subPCP/M100907, 0.31 mg/kg), n = 9 (subPCP/buspirone, 1.1 mg/kg). (A) **P < 0.01; ***P < 0.001 versus familiar object exploration. (B) ***P < 0.001 versus discrimination index in the SubVeh/vehicle group; *P < 0.05, **P < 0.01; ***P < 0.001 versus discrimination in the subPCP/vehicle group.

**Fig. 3.** Effects of brexpiprazole (BREX) alone and in combination with WAY100635 and of M100907 on (A) exploration time(s) of familiar and novel objects and (B) discrimination index in a NOR task in rats treated with subchronic PCP. M100907 and WAY100635 were administered subcutaneously 30 minutes before acquisition trial. Brexpiprazole was administered p.o., 2 hours before acquisition trial. Data represent mean ± S.E.M., n = 12 (subVeh/vehicle), n = 10 (subPCP/vehicle), n = 9 (subPCP/M100907, 0.013 mg/kg), n = 6 (subPCP/M100907, 0.31 mg/kg), n = 10 (subPCP/brexpiprazole, 3.0 mg/kg), n = 11 (subPCP/brexpiprazole, 3.0 mg/kg + WAY100635, 0.5 mg/kg). (A) *P < 0.05; **P < 0.01; ***P < 0.001 versus familiar object exploration. (B) *P < 0.05 versus SubVeh/vehicle group; *P < 0.05 versus SubPCP/vehicle group; #P < 0.05 versus SubPCP/vehicle group.
Results

Brexpiprazole Has Antipsychotic-Like Activity in Rats

CAR. Brexpiprazole induced a dose-dependent inhibition of CAR with an ED_{50} value of 6.0 mg/kg p.o., similar to risperidone (ED_{50} = 3.3 mg/kg p.o.; Table 1). Aripiprazole also inhibited CAR in a dose-dependent manner with an ED_{50} value of 23 mg/kg p.o., which is significantly less potent than that of brexpiprazole. Moreover, although brexpiprazole did not induce any nonspecific escape failures at the dose range tested (1.5–12 mg/kg p.o.), aripiprazole and risperidone induced escape failure at a rate of 5 and 30% at the highest doses used (60 and 12 mg/kg p.o.), respectively (data not shown).

Apomorphine-Induced Hyperactivity and Stereotyped Behavior. Brexpiprazole dose-dependently inhibited apomorphine-induced locomotor hyperactivity with an ED_{50} value of 2.3 mg/kg p.o. and apomorphine-induced stereotyped behavior with an ED_{50} value of 2.9 mg/kg p.o. (Table 1). In the same tests, aripiprazole was found to be slightly less potent than brexpiprazole, with ED_{50} values of 3.2 (locomotor hyperactivity) and 6.1 (stereotyped behavior) mg/kg. With similar potency to brexpiprazole, risperidone also inhibited apomorphine-induced stereotyped behavior with an ED_{50} value of 4.7 mg/kg p.o. (Table 1).

Amphetamine-Induced Hyperactivity. The difference in potency of the inhibitory effects of brexpiprazole and aripiprazole against hyperactivity induced by D-amphetamine (ED_{50} = 0.92 and 3.9 mg/kg, respectively) paralleled the results obtained with apomorphine. The inhibitory effect of brexpiprazole was more potent than that of aripiprazole (Table 1).

Inhibition of Spontaneous Locomotor Activity. Like other D_{2} partial agonists and D_{3} antagonists, brexpiprazole (ED_{50} = 3.4 mg/kg p.o.) and aripiprazole (ED_{50} = 6.1 mg/kg p.o.) inhibited spontaneous locomotor activity at slightly higher than or similar doses at which they inhibited the dopaminergic stimulant-induced behaviors (Table 1).

Catalepsy. Catalepsy was observed only at high doses of brexpiprazole and aripiprazole, with ED_{50} values of 20 and 42 mg/kg p.o., respectively (Table 1). The dose ratios of cateleptogenic activity against the inhibitory effect on dopaminergic stimulant-induced behavior and CAR tests were 3.3–21.7 for brexpiprazole and 1.8–13.1 for aripiprazole, respectively. In comparison, dose ratios of risperidone were 1.4–2.0 (Table 1).

Brexpiprazole Inhibits Apomorphine-Induced Eye Blinking in Monkeys

Apomorphine induces a characteristic eye blinking response in Cynomolgus monkeys (Table 2). Brexpiprazole potently antagonizes the apomorphine-induced eye blinking response with an ED_{50} value of 0.03 mg/kg (95% CI; 0.0004–0.08 mg/kg p.o.; for individual data, see Table 2).

Brexpiprazole Reverses Cognitive Deficits Induced by Subchronic PCP Treatment

Subchronic PCP treatment is known to impair cognitive performance in various tests (Rodefer et al., 2008; Neill et al., 2010). Two tests were selected for characterization of brexpiprazole and aripiprazole in the present studies: NOR and ID-ED.

TABLE 1
Overview of effects in rat test models of positive psychotic symptoms of schizophrenia and CNS side effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>No. in Group</th>
<th>Brexpiprazole (1 h)a</th>
<th>Aripiprazole (2 h)b</th>
<th>Risperidone (1 h)c</th>
<th>mg/kg p.o. (95% CI)</th>
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<tbody>
<tr>
<td>Efficacy tests</td>
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<tr>
<td>CAR</td>
<td>4–6</td>
<td>6.0 (4.3–9.7)</td>
<td>23 (20–27)</td>
<td>3.3 (2.0–7.6)</td>
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<tr>
<td>APO hyperactivity (0.25 mg/kg)</td>
<td>5</td>
<td>2.3 (1.2–3.1)</td>
<td>3.2 (1.9–5.0)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>APO stereotypy (0.7 mg/kg)</td>
<td>6</td>
<td>2.9 (2.2–3.8)</td>
<td>6.1 (5.2–7.2)</td>
<td>4.7 (2.9–6.9)</td>
<td></td>
</tr>
<tr>
<td>AMPH hyperactivity (0.5 mg/kg)</td>
<td>8</td>
<td>0.92 (0.6–1.5)</td>
<td>3.9 (2.0–7.7)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>CNS side effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibition of spontaneous locomotor</td>
<td>8</td>
<td>3.4 (2.4–5.0)</td>
<td>6.1 (2.3–16)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>activity at time of maximum effect</td>
<td>6</td>
<td>20 (12–40)</td>
<td>42 (26–68)</td>
<td>6.6 (2.9–16)</td>
<td></td>
</tr>
</tbody>
</table>


AMPH, amphetamine; APO, apomorphine; NT, not tested.

aExcept for spontaneous locomotor activity, amphetamine-induced hyperactivity (2 hours) and catalepsy (maximum effect at 6 hours).

bExcept for catalepsy (maximum effect at 8 hours).

cExcept for catalepsy (2 hours).
NOR. The test was performed in three separate experiments (Figs. 1–3). During the 3-minute test trial in each experiment, the subVeh rats, treated acutely with vehicle on the test day, spent significantly more time exploring a novel object than a familiar object (P < 0.001). In contrast, subPCP animals, treated acutely with vehicle on the test day, demonstrated significant impairment of NOR by spending approximately equal amounts of time exploring both a familiar and a novel object.

In the first experiment, brexpiprazole at doses of 0.3, 1.0, and 3.0 mg/kg p.o., and aripiprazole at a single high dose of (10 mg/kg p.o.) were studied. As shown in Fig. 1A (exploration time) and Fig. 1B (discrimination index, i.e., time spent exploring the novel versus familiar object, adjusted for total exploration time), acute administration of brexpiprazole (1.0 and 3.0 mg/kg p.o.) significantly attenuates subPCP-induced deficits in exploration (P < 0.001 and P < 0.01, respectively) and in discrimination index (P < 0.001 and P < 0.01, respectively). In contrast, aripiprazole (10 mg/kg p.o.) was ineffective. Neither brexpiprazole nor aripiprazole significantly affected total object exploration time at any dose tested (data not shown).

In the second experiment, the effect of brexpiprazole was compared with those of the 5-HT<sub>2A</sub> antagonist M100907 and the 5-HT<sub>1A</sub> partial agonist buspirone, as shown in Fig. 2, A and B. Acute administration of brexpiprazole (3.0 mg/kg p.o., M100907 (0.31 mg/kg s.c.), and buspirone (1.1 mg/kg s.c.) attenuated subPCP-induced deficits in exploration, with statistical significance of P < 0.001, P < 0.01, and P < 0.001, respectively (Fig. 2A). Aripiprazole (20 mg/kg p.o.) was also included in this experiment, but less than half of the group (only four rats) complied with the test criteria; accordingly, the result was considered inconclusive and is not shown. However, plasma concentration of aripiprazole at that time was measured (Table 3). As shown in Fig. 2B, the subPCP-induced decrease in discrimination index is fully reversed by brexpiprazole at 3.0 mg/kg p.o. (P < 0.001), and partially but significantly attenuated by M100907 (0.31 mg/kg s.c., P < 0.01) and buspirone (1.1 mg/kg s.c., P < 0.05). At the doses tested, none of the drugs had any significant effects on total object exploration time (data not shown).

In the third experiment, the goal was to extend the dose range studied for M100907 and to explore whether the 5-HT<sub>1A</sub> antagonist WAY100635 would attenuate the effect of brexpiprazole. Figure 3A shows that subPCP-induced deficits in novel object exploration were significantly attenuated by M100907 (0.013 and 0.31 mg/kg s.c., P < 0.01 and P < 0.01, respectively), brexpiprazole (3.0 mg/kg p.o., P < 0.001), and brexpiprazole (3.0 mg/kg p.o.) coadministered with WAY100635 (0.5 mg/kg s.c., P < 0.05). Also, as shown in Fig. 3B, the decrease in discrimination index induced in the subPCP group was significantly attenuated after acute administration of brexpiprazole (3.0 mg/kg p.o., P < 0.05). In contrast, acute administration of M100907 (0.013 and 0.31 mg/kg s.c.) failed to significantly reverse discrimination index, although a numeric reversal was shown. Finally, when brexpiprazole (3.0 mg/kg p.o.) was coadministered with WAY100635 (0.5 mg/kg s.c.), it no longer reversed the subPCP-induced decrease in discrimination index. Total exploration time was significantly increased in the third experiment for brexpiprazole (3.0 mg/kg p.o.) and M100907 (0.013 mg/kg s.c.), compared with the subPCP-Veh group (P < 0.05), whereas M100907 (0.31 mg/kg s.c.) and brexpiprazole (3.0 mg/kg p.o.) cotreatment with WAY100635 (0.5 mg/kg s.c.) had no significant effect on object exploration time (data not shown).

Plasma Exposure. After completion of the NOR experiments—about 3 hours after administration of compounds—plasma concentrations of brexpiprazole and aripiprazole were measured. Mean plasma levels of brexpiprazole were 11, 31, and 74–113 (range based on three experiments) ng/ml at 0.3, 1.0, and 3.0 mg/kg p.o., respectively (Table 3). The mean plasma levels of aripiprazole were 40 and 158 ng/ml at 10 and 20 mg/kg p.o., respectively.

ID-ED. Figure 4 shows that subPCP treatment leads to a specific impairment in ED set-shifting 1 week after PCP withdrawal. The impairment is measured as an increased

### Table 2

<table>
<thead>
<tr>
<th>Effect</th>
<th>Total Counts of Eye Blinking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 1</td>
<td>Animal 2</td>
</tr>
<tr>
<td>Spontaneous eye blinking</td>
<td>65 ± 4.5</td>
</tr>
<tr>
<td>Apomorphine-induced eye blinking</td>
<td>112 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inhibitory effect of brexpiprazole (mg/kg p.o.) on apomorphine-induced eye blinking</td>
<td>0.03</td>
</tr>
<tr>
<td>0.1</td>
<td>59</td>
</tr>
<tr>
<td>0.3</td>
<td>40</td>
</tr>
<tr>
<td>1.0</td>
<td>19</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.05, statistically significant compared with spontaneous eye blinking (paired t test, two-tailed).

<sup>b</sup>P < 0.01, statistically significant compared with spontaneous eye blinking (paired t test, two-tailed).

### Table 3

<table>
<thead>
<tr>
<th>Treatment (Dose)</th>
<th>Plasma Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td>mg/kg p.o.</td>
<td>ng/ml ± S.E.M.</td>
</tr>
<tr>
<td>Brexpiprazole</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>11 ± 1.3</td>
</tr>
<tr>
<td>1.0</td>
<td>31 ± 2.7</td>
</tr>
<tr>
<td>3.0</td>
<td>113 ± 36</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>40 ± 1.5</td>
</tr>
<tr>
<td>20</td>
<td>—</td>
</tr>
</tbody>
</table>
number of trials to learn the ED task compared with rats treated with subchronic saline \((P < 0.01)\). Furthermore, this confirms the formation of an attentional set shifting as a validation criterion for the test because the control rats treated with subVeh needed significantly more trials to learn the ED task than the preceding task, the ID2 task \((P < 0.05)\).

Brexpiprazole significantly reversed the subPCP-induced impairment in the ED task at a dose of 1.0 mg/kg s.c., whereas the decrease in the number of trials needed to reach the criterion measured at a higher dose (3.0 mg/kg s.c.) was not statistically significant (Fig. 4). Aripiprazole (10 mg/kg s.c.) did not alter the PCP-induced impairment. Interestingly, the reversal of the PCP-induced impairment by the reference drug modafinil (64 mg/kg p.o.) did not reach statistical significance \((P = 0.053)\). In addition to its effect on the ED stage performance, brexpiprazole (1.0 mg/kg s.c.) also significantly increased the number of trials needed to reach criterion in the EDR.

Discussion

Brexpiprazole, a novel serotonin-dopamine activity modulator, was developed and optimized to achieve clinical efficacy with minimal EPS potential. Its target optimization included D2 partial agonism with low intrinsic activity, 5-HT2A partial agonism, and antagonism at 5-HT2A, α1B-, and α2C-adrenergic receptors (Maeda et al., 2014), supporting a favorable antipsychotic profile with low EPS risk and potential to treat core symptoms in schizophrenia, including cognitive deficits (Arnt and Skarsfeldt, 1998). Brexpiprazole showed antipsychotic-like activity in tests established for efficacy of D2 antagonists on positive symptoms (Table 1), and efficacious doses show D2 receptor occupancies within the clinically-relevant dose range for treatment of schizophrenia (Maeda et al., 2014). The inhibitory activity of brexpiprazole on drug-induced hyperactivity is slightly more potent than that on spontaneous locomotor activity, suggesting specific antipsychotic-like efficacy.

Classic animal tests of positive psychotic symptoms are usually unable to reveal differences between antipsychotic drugs (Arnt and Skarsfeldt, 1998). In contrast, the catalepsy test differentiates among antipsychotics to predict EPS potential. Unlike haloperidol, both aripiprazole (Kikuchi et al., 1995) and risperidone (Arnt and Skarsfeldt, 1998) have shown separation between antipsychotic-like and cataleptogenic activity. Although the potency of brexpiprazole to induce antipsychotic-like activity \((ED_{50} = 0.92–6.0 \text{mg/kg})\) is in the same range as that predicting in vivo D2 receptor occupancy \((ED_{50} = 2.5 \text{mg/kg p.o.}; \) Maeda et al., 2014), cataleptogenic activity is only induced at high dosages \((ED_{50} = 20 \text{mg/kg})\), likely due to D2 partial agonism and its 5-HTergic activities (Kikuchi et al., 1995; Meltzer 1999). Moreover, the potent inhibition by brexpiprazole of the apomorphine-induced eye blinking in monkeys is consistent with a low intrinsic activity at D2 receptors because D2 partial agonists with moderate intrinsic activity increase the eye-blinking rate (Kleven and Koek, 1996) whereas D2 antagonists have inhibitory effects (Karson, 1983; Elsworth et al., 1991).

A major unmet medical need in the treatment of schizophrenia is improvement of cognitive dysfunction. Cognitive function has been identified as the primary factor determining functional outcome for schizophrenia patients, and efficacies of present treatments are limited (Green, 1996; Keefe and Harvey, 2012; Keefe et al., 2013). In recent years, focus on cognitive domains has increased (e.g., the MATRICS initiative [Measurement and Treatment Research to Improve Cognition in Schizophrenia]; Green et al., 2004; Young et al., 2009), and several animal models have been developed to improve the translational predictability to clinical effects.

The most widely used models presently apply either subPCP or subchronic ketamine, inducing enduring cognitive impairment across several cognitive domains (Neill et al., 2010; Nikiforuk and Popik, 2012). SubPCP-induced cognitive deficits are associated with changes in brain function, resembling many of the changes in patients with schizophrenia (Neill et al., 2010). Furthermore, the subPCP model has the advantage that evaluation of the effects of test compounds are conducted after withdrawal of PCP, thus avoiding risk for confounding effects through drug-drug interactions with the tool compound (PCP).

In the present study, brexpiprazole and aripiprazole have been studied in two different cognitive tests, the NOR and the ID-ED tests, which are regarded as relevant for evaluating cognitive deficits in schizophrenia and other CNS disorders and require episodic memory and executive function (problem solving; cognitive flexibility), respectively (Green et al., 2004; Young et al., 2009).

Brexpiprazole fully reversed subPCP-induced impairments in the NOR test at 1.0–3.0 mg/kg with good reproducibility. These doses are within the dose range for antipsychotic-like efficacy (Table 1), suggesting that positive symptoms and cognitive impairment can be treated within a similar dose range. By comparing exposure analyses with the in vivo binding data in the adjoined paper (Maeda et al., 2014), receptor occupancies are estimated at 35% (1.0 mg/kg) and 64–76% (3.0 mg/kg) for D2 receptors and 25% (1.0 mg/kg) and 45–55% (3.0 mg/kg) for 5-HT2A receptors, respectively. In addition, a moderate to high occupancy for 5-HT1A receptors is also predicted, based on the high in vitro affinity for this receptor (5-HT1A ≥ D2 = 5-HT2A) and the potency obtained by the ex vivo binding study (Maeda et al., 2014).

In contrast to brexpiprazole, and in spite of sufficient plasma exposure at clinically relevant doses of aripiprazole (10 and 20 mg/kg) predicting high D2 receptor occupancies...
5-HT2A receptors are contributors. The 5-HT1A partial agonist 5-HT2A receptors were estimated to be much lower (4–18% for 5-HT2A receptors at 10–20 mg/kg; Maeda et al., 2014). Pharmacological analyses of targets involved in brexpiprazole efficacy in the NOR test suggest that both 5-HT1A and 5-HT2A receptors are contributors. The 5-HT1A partial agonist buspirone had a significant effect (though did not fully reverse the subPCP deficit), and the 5-HT1A antagonist WAY100635 partially, but significantly, reversed the effect of brexpiprazole. Similar to buspirone, the selective 5-HT2A antagonist M100907 had partial effects at a dose range displaying 5-HT2A receptor occupancies of 50% and higher (Idris et al., 2010). It is unlikely that D2 partial agonism is an essential effect, as aripiprazole was ineffective, despite a similar D2 occupancy to brexpiprazole. Other supportive data have suggested 5-HT1A agonism as the preferential target reversing subPCP-induced cognitive impairment, in particular the results of experiments in which buspirone and another 5-HT1A partial agonist, tandospirone, were also effective (Horiguchi et al., 2012; Horiguchi and Meltzer, 2012). It has also been suggested that 5-HT2A blockade contributes to the ability of atypical antipsychotics to improve impairment of NOR caused by subPCP treatment (Meltzer et al., 2011). However, the contribution of 5-HT2A antagonism is ambiguous; some studies show efficacy levels comparable with those of the present study, but others do not (Grayson et al., 2007; Redrobe et al., 2010; Meltzer et al., 2011; Horiguchi et al., 2012).

Brexiprazole also showed significant procognitive activity in the ID-ED test model of executive function in subPCP-treated rats whereas aripiprazole was again ineffective at the dose tested. The effect is a selective improvement of ID-ED–shift task performance. Notably, brexpiprazole (1.0 mg/kg) also increased trials to criterion in the EDR stage. The cause of this finding is currently unknown, but it could be a result of decreased motivation to perform this stage of the task. Importantly, the decreased EDR performance found here is not considered to be a general effect on reversal learning because no effect of brexpiprazole was found on the ID2R stage. This test model has shown ability to differentiate among antipsychotics with different pharmacological profiles. It has been reported that haloperidol is ineffective whereas risperidone, olanzapine, and clozapine show nonsignificant trends toward improved ED-shift performance, and other antipsychotics, serindole, and quetiapine show full reversal (Rodefer et al., 2008; Goethebeur and Dias, 2009; Nikiforuk and Popik, 2012). In addition, studies of selective 5-HT receptor subtype antagonists indicate that the 5-HT2A antagonist M100907 has borderline effects similar to risperidone whereas a 5-HT6 antagonist [SB-271046, 5-chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[b][1,4]thiazepine-791 DOI: 10.1358/dof.2008.033.09.1236966.]

Contrary to our results with aripiprazole, more positive results have been reported elsewhere. In subPCP-treated mice in the NOR test, aripiprazole reversed deficits, which were prevented by cotreatment with WAY100635, suggesting mediation by 5-HT1A receptors (Nagai et al., 2009). The reason for the lack of efficacy in this study is unknown, but may be due to differences of species, experimental design, and pharmacologic profile. In summary, the present study suggests that brexpiprazole is a promising novel antipsychotic drug with robust efficacy on positive symptoms and cognitive impairment, with a favorable CNS safety profile in animal models. This is probably due to its broad serotonin-dopamine-system modulating activity as well as its antagonism of relevant noradrenergic receptors.

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Performed data analysis: Maeda, Sugino, Akazawa, Amada, Lerdup, Bundgaard.
Wrote or contributed to the writing of the manuscript: Maeda, Arnt, Lerdup, Bundgaard, Stensbøl.

References